FORM PTO-1390 (Modified) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE ATTORNEY'S DOCKET NUMBER 14 SEP 1998
(REV 5-93)

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

60953/119

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)
09/142660

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE97/00494	**March 12, 1997**	**March 14, 1996**

TITLE OF INVENTION
DETECTION OF MOLECULES AND MOLECULE COMPLEXES

APPLICANT(S) FOR DO/EO/US
Rainer HINTSCHE and Manfred PAESCHKE

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☒ A translation of the International Application into English (35 U.S.C. 371 (c)(2)).

7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

10. ☒ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.

14. ☐ A substitute specification.

15. ☐ A change of power of attorney and/or address letter.

16. ☐ Other items or information:

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.50	INTERNATIONAL APPLICATION NO. PCT/DE97/00494	ATTORNEY'S DOCKET NUMBER 60953/119

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5):		
Search Report has been prepared by the EPO or JPO **$930.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) . . . . . **$.00**		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2) . . . . . **$.00**		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO . . . . . **$.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) . . . . . **$.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 930.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e))	$ 0.00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	**20** -20 =	**0**	**X $22.00**	$ **0.00**	
Independent Claims	**1** -3 =	**0**	**X $82.00**	$ **0.00**	
Multiple dependent claim(s) (if applicable)			**+ $270.00**	$ **0.00**	
TOTAL OF ABOVE CALCULATIONS =				$ **930.00**	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$ **0.00**	
SUBTOTAL =				$ **930.00**	
Processing fee of **$130.00** for furnishing English translation later the ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f). +				$ **0.00**	
TOTAL NATIONAL FEE =				$ **930.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ **0.00**	
TOTAL FEES ENCLOSED =				$ **930.00**	
				Amount to be: refunded $	
				charged $	

a. ☒ A check in the amount of **$930.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 19-0741 in the amount of $ to the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0741. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Foley & Lardner
3000 K Street, N.W., Suite 500
P.O. Box 25696
Washington, D.C. 20007-8696

SIGNATURE

Colin G. Sandercock
NAME

31,298
REGISTRATION NUMBER

Form PTO-1390 (REV 5-93) (Modified)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 060953/0119

In re patent application of

Rainer HINTSCHE and Manfred PAESCHKE

Filed: September 14, 1998

U.S. National Phase PCT/DE97/00494
International Filing Date: March 12, 1997

For: DETECTION OF MOLECULES AND MOLECULE COMPLEXES

SECOND PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend the above captioned application as follows:

In the Claims

1. [Twice Amended] [Method for the detection of molecules or molecule complexes in a diluent or solvent,

- a sample to be measured being brought into contact with an ultra-microelectrode arrangement which has at least two electrode structures that are applied to or incorporated in an insulating support material and that are uncovered in the direction of the measurement and arranged relative to one another such that the distances between the various structures lie in the ultra-micro range,

- an alternating electric field being produced by application of an electric potential, and

- the changes in current or potential, which are caused by species present or created in the sample to be measured, being measured.]

Method for the detection of molecules or molecule complexes in a diluent or solvent comprising,

providing a sample to be measured,

bringing the sample into contact with an ultra-microelectrode arrangement which has at least two electrode structures that are applied to or incorporated in an insulating support material and that are uncovered in the direction of the measurement and arranged relative to one another such that the distances between the various structures lie in the ultra-micro range,

producing an alternating electric field by application of an electric potential, and

measuring the changes in current or potential, which are caused by species present or created in the sample to be measured.

17. [Twice Amended] [Method according to one of claims 1-16, in which the insulating support material is selected from silicon compounds, glass, ceramic, organic polymers.]

Method according to claim 1, wherein the insulating support material is selected from the group consisting of silicon compounds, glass, ceramic or organic polymers.

Respectfully submitted,

December 22, 1998
Date

Colin G. Sandercock
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 060953/0119

In re patent application of

Rainer HINTSCHE and Manfred PAESCHKE

Filed: September 14, 1998

U.S. National Phase PCT/DE97/00494
International Filing Date: March 12, 1997

For: DETECTION OF MOLECULES AND MOLECULE COMPLEXES

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend this application as follows:

In the Specification

At page 14, line 29, please insert the following:

--It will be apparent to those skilled in the art that various modifications and variations can be made to the methods of this invention. Thus, it is intended that the present invention cover such modifications and variations, provided they come within the scope of the appended claims and their equivalents.

The disclosure of all publications cited above are expressly incorporated herein by reference in their entireties to the same extent as if each were incorporated by reference individually.

The disclosure of the German Patent Application No. 196 10 115.8, for which benefit under 35 USC §119 is claimed, is expressly incorporated by reference herein in its entirety, including the specification, claim and figures.

The disclosure of the PCT Application No. PCT/DE97/00494, for which benefit under 35 USC §119 is claimed, is expressly incorporated by reference herein in its entirety, including the specification, claim and figures.

The amendments of May 2, 1998 to the specification and claims under Article 34 of the PCT Application No. PCT/DE97/00494, also are expressly incorporated by reference herein in their entirety.—

In the Claims

Please amend the following claims. Amended claims 1-5 are presented for examination as further amended herein.

Claim 3, line 1; delete "or 2"

Claim 4, line 1; change "one of claims 1 to 3" to –claim 1--

Claim 5, line 1; change "one of claims 1 to 4" to –claim 1--

Claim 6, line 1; change "one of claims 1 to 5" to – claim 1--

Claim 8, line 1; change "one of claims 1 to 7" to –claim 1--

Claim 9, line 1; change "one of claims 1 to 8" to –claim 1--

Claim 10, line 1; change "one of claims 1 to 9" to –claim 1--

Claim 11, line 1; change "one of claims 8 to 10" to –claim 8--

Claim 13, line 1; delete "or 12"

Claim 15, line 1; change "one of claims 1 to 14" to –claim 1--

Claim 16, line 1; change "one of claims 1 to 15" to -claim 1—

Claim 17, line 1; change "one of claims 1 to 16" to -claim 1--

Claim 18, line 1; change "one of claims 1 to 17" to -claim 1--

Claim 19, line 1; change "one of claims 1 to 18" to -claim 1--

Claim 20; line 1; change "one of claims 1 to 19" to -claim 1--

Respectfully submitted,

Sept. 14, 1998
Date

for Colin G. Sandercock (28,665)
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Applicant or Patentee: Rainer Hintsche and Manfred Paeschke
Serial or Patent No.: 09/142,660 Atty. Dkt. No. 060953/0119
Filed or Issued: September 14, 1998
For: DETECTION OF MOLECULES AND MOLECULE COMPLEXES

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) AND 1.27 (c)) — SMALL BUSINESS CONCERN

I hereby declare that I am
( ) the owner of the small business concern identified below:
( ) an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN FRAUNHOFER-GESELLSCHAFT ZUR FOERDERUNG DER ANGEWANDTEN FORSCHUNG E.V.
ADDRESS OF CONCERN Leonrodstrasse 54, D-80636 Muenchen, Germany

I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18 and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention, entitled DETECTION OF MOLECULES AND MOLECULE COMPLEXES by inventor(s) Rainer Hintsche and Manfred Paeschke described in

( ) the specification filed herewith
(xx) application serial no. 09/142,660, filed September 14, 1998
( ) patent no. _____, issued _____

If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e). * NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities: (37 CFR 1.27)

NAME: _____
ADDRESS: _____
( ) INDIVIDUAL ( ) SMALL BUSINESS CONCERN ( ) NONPROFIT CORPORATION

NAME: _____
ADDRESS: _____
( ) INDIVIDUAL ( ) SMALL BUSINESS CONCERN ( ) NONPROFIT CORPORATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate: (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING: Helmut Schubert
TITLE OF PERSON OTHER THAN OWNER: Head of Patent Department
ADDRESS OF PERSON SIGNING: Zeppelinstraße 81, D-81669 München
SIGNATURE: _____ DATE: 3rd December 1998

Detection of molecules and molecule complexes

The invention relates to a method for the detection of molecular species, and to an electronic sensor therefor. Electronic sensors of this type, also referred to as ultra-microelectrode arrays, can be used for chemical analysis and process control in a variety of fields, such as the health service, biotechnology, environmental protection and the chemical industry. They represent a comparatively simple measuring system which measurably registers the binding or attachment of molecules in the area close to the electrodes.

Hitherto known are optical sensors which make it possible to detect binding effects or the attachment of molecules in thin layers, amongst other things, using the **evanescent wave** [cf. Feldman et al., Biosens. & Bioelectron., 10 (1995) 423] or **light-reflection** [cf. Domenici et al., Biosen. & Bioelectron., 10 (1995) 371 or Brecht, Gauglitz, Biosen. & Bioelectron., 10 (1995), 923] or **surface plasmon resonance** [cf. Häuseling et al., Langmuir, 7 (1991) 1837 or U. Jönsson et al., BioTechniques 11 (1991), 620] principles.

For the direct electrical reading of binding events of this type, a potentiometric measurement method [cf. Bergfeld, Biosen. & Bioelectron., 6 (1991), 55], a capactive measurement method [cf. Swietlow, Electroanalysis, 4 (1992), 921] and an impedimetric

measurement method [cf. Knichel et al. Sens. & Act. B 28, (1995), 85] have already been described. Electrode arrangements based on the EIS principle (EIS: Electrolyte-insulator-semiconductor) have also been proposed [cf. Schyberg et al. Sens. & Act. B 26-27 (1995) 457 or Souteyrand et al. Sens. & Act. B 20, (1994) 63], the insulator acting as a coupling and relay element.

In these electrochemical or measurement arrangements, electrodes that are spatially far removed from one another are used to register molecules in the thin boundary layer close to the electrodes, but these are negatively affected in a variety of ways by a comparatively large amount of electrolytes and other substances between the electrodes.

Applications are also known in which thin molecular layers have been deposited as a gate between the drain and source of transistors and provide information regarding the organic layer [cf. Kruse et al. Sens. & Act. B 6 (1992), 101 or Uhe et al. Electroanalysis, 6 (7) (1994), 543].

A fact common to all these described electrical methods with electrodes is that they do not have any arrangements approaching molecular dimensions; in all of these applications, the lengths typical of the sensors, for example between the measuring, reference and working electrodes, are orders of magnitude away

from molecular dimensions.

The **object of the invention** is to provide a method using an electronic sensor which permits the detection of molecules and molecule complexes at higher detection sensitivity with comparatively lower system outlay.

The way in which this object is achieved according to the invention is described in claim 1. The further claims present preferred refinements.

According to the invention, the method for the detection of molecules and molecule complexes is carried out with an arrangement which has an ultra-microelectrode array whose electrode structures are arranged so closely next to one another that they approach the size of large molecule complexes, for example immunoproteins or DNA molecules. Use is, in particular, made of the effect that it is possible for alternating electric fields to be produced between closely neighboring electrodes and the resulting current is predominantly affected by the detected molecules and molecule complexes in the area close to the electrodes. There is in this case a relatively free choice over the shape and fine structure of the electrodes, while the minimum spacing of the electrodes themselves should typically be less than 3 µm, preferably 1 µm.

The way in which the current is affected may

involve diffusion, attachment or binding of the species to be measured. Through this way of generating the field and of taking measurements, in particular using impedance spectroscopy, the invention achieves the result that electrolyte molecules and other substances in a sample to be measured have only a slight effect on the electric field existing between the electrodes, and do not therefore interfere with the measurement.

A multiple arrangement of this kind of fine-structured ultra-microelectrode array advantageously leads to amplification of the effect described above, in which measurements of the same type are taken sequentially or in parallel using a suitable measuring technique (for example impedance measurement bridges). The ultra-microelectrode arrays may consist of thin layers of noble metals such as gold, platinum or iridium, or alternatively carbon materials, or may contain these materials (claim 16). They are particularly advantageously applied to planar insulating support materials such as silicon compounds, glass, ceramic or organic polymers, but may also, for planarization and mechanical support, be buried or incorporated in these materials (claim 17). Two mutually insulated ultra-microelectrodes can be brought together optimally, as represented in Fig. 1, for example using bands or parallel strips or meandering and round or coiled structures, as well as using

finger-like interdigital arrangements at distances of preferably less than 1 µm. In relation to this, Fig. 1 gives arrangement examples a to d (see below). The electrodes are preferably uncovered in the direction of the measurement area.

One particular refinement of the arrangement of the ultra-microelectrode array which may be provided is to stack an electrode array with one or more others and to insulate the crossover points from one another using insulation layers (claim 19). It is in this way possible for the electrodes to be arranged at distances of only a few nm from one another, with the insulation layer defining the minimum spacing (Fig. 1e). One fact common to all the ultra-microelectrode array arrangements is that they must be properly insulated from one another so that two, three or more ultra-microelectrode arrays can have direct and/or alternating current applied to them individually or in groups, electrically independently through an insulated supply lead on the chip (claim 20). The materials used for the insulation (for example plastics or inorganic compounds such as silicon oxides, nitrides and ceramic materials) need to be inert over the working period with respect to the diluents or solvents (often water) used in the sample. The term "solvent" is intended to mean reaction liquids in which it is possible for the molecules to bind, become attached or diffused. The

sample to be measured need not, however, necessarily be liquid, and other states are also possible. The processes to be measured may thus also take place in a gel.

Between the ultra-microelectrodes, the electric field employed for detection may be produced by alternating current with frequencies of between 1 mHz and 10 MHz and amplitudes of about 10 mV and 50 mV. In this case, potentials of between 0 V and +/-5 V are chosen.

The present method even makes it possible to register complex reaction processes, and therefore affords enhanced possibilities for use. The penetration of molecules into the region close to the electrodes with the field which is built up (for example by diffusion) or the arrangement of molecules in this region, which may for example take place through so-called "self assembling" or else through complexing, alter both the real and imaginary parts of the complex impedance, and may be measured independently of time - for example after the events have ceased to take place - as well as with the phase angle, if necessary, but it may also be measured as a function of time, that is to say on the basis of the progress of the binding event or the diffusion (claims 3 and 4). For a complete impedance spectrum, the entire frequency range is measured and evaluated. The use according to the

invention of only individual selected frequencies or frequency ranges, which are maximally affected, is particularly advantageous. This makes it possible to design miniaturized detection systems.

When use is made of the ultra-microelectrode arrays in liquids or the like, it is also possible, in addition to their measuring process - or alternatively in pauses between measurements - for direct-current components to be superimposed or applied (claim 6). These may, for example, induce electrochemical reactions such as oxidations or reductions of electrically active molecules, with processes of this type being measured simultaneously or sequentially with the impedance measurements (claim 7). According to the invention, this permits a combination of electrical and electrochemical measurements with the same sensor arrangement (ultra-microelectrode array).

According to the invention, the method may be carried out for the detection of molecules and molecule complexes by making the molecules which are to be measured bind to the actual microelectrode surfaces. This binding may be physical (adsorption) or chemical. For the latter case, the self-assembling methods are particularly well-suited, which make it possible, for example, to bind monomolecular thiol compounds on gold electrodes and measure them. This method is universally applicable for a large number of molecules, and not

only for those which have, or may be provided with, a thiol group.

A second selective method for making molecules and molecule complexes adhere to the conductive microelectrode surfaces is the known method of electropolymerization (claim 9). In this case, each electrode may be modified individually, in groups or in parallel, on its surface with electropolymers, for example made up of the monomer molecules streptavidin, pyrrole, aniline, vinyl ferrocene or other electrically polymerizable substances. The binding of compounds of this type in monomolecular or multimolecular layers on the electrodes changes the impedance spectrum or individual frequencies in a very characteristic fashion, and can therefore be measured as a function of time or after completion of the reaction.

Further, the impedance spectrum may also be measurably changed if the molecules are positioned in the gaps between electrodes instead of on the electrodes (claim 10). This positioning may be carried out, for example, by chemical binding (for example to silicon dioxide) or by adhesion or by reactions such as condensation reactions, for example silanizing. In order to coat the entire surface of the electrode array, that is to say the electrodes themselves as well as the gaps between electrodes, the known Langmuir-Blodget method may be employed (Tachibana Matsumoto,

Advanced Materials Ab. 11 (1993), 5/796-803) with which, for example, lipids or phthalocyanines can be arranged in layers by pulling monomolecular films.

According to a further variant of the method according to the invention for the detection of molecules and complexes, the concentration of molecules in the layer close to the electrodes may be altered by diffusion, and the alteration may be measured. This can be done both using chemically/physically related changes in concentration, and by applying an electric potential which produces a diffusion gradient. It is further possible to bring about and measure the production of specific molecules, for example by enzymes, in the area close to the electrodes.

According to the invention, in a preferred refinement, the method for the detection of molecules and molecule complexes comprises the measure that the molecular layers produced beforehand on the electrode arrays are or will be provided with chemical bonding groups that can bind further molecules by a chemical reaction or complexing (claim 11). It is in this way possible to monitor binding events of this type with high sensitivity. If, for example, a complexing agent of low molecular weight such as biotin is bound to the electrode via a thiol functional group, then this biotin may subsequently be complexed with a complexing partner of fairly high molecular weight, for example

streptavidin, to which an arbitrary number of further molecules can be bound.

One particularly important and very widely usable application of the present invention is immunodetection (claim 12). In this case, molecular layers are built up on the ultra-microelectrode array using the sandwich principle of an antibody/antigen immune reaction. In order to detect antibodies in the sample to be measured, haptens (antigens with low molecular weight) or other antigens (often proteins), for example, may for this purpose be bound to the microelectrode arrays. In this way, the specific complexing between the firmly anchored antigens and the antibodies found in the sample to be measured lead to specific antibody detection. In reversal of this principle, it is also possible for the antibodies to be bound to the electrodes and for haptens or the like to be detected from the sample to be measured. The antigen may also be a virus protein with fairly high molecular weight, which is firmly bound to the microelectrode array and makes it possible to measure antibodies from the sample to be measured. Variants of this method include the use of polyvalent antibodies with which it is possible to construct and measure threefold or higher molecule complexes.

A further refinement of the method according to the invention is provided if the ultra-microelectrode

array is used for the electrical reading of hybridization processes in nucleic acid chemistry (claim 13). Applications in genetic engineering can be produced by binding nucleotides via thiol bonds or the like to the electrode structures and registering the binding of complementary nucleic acid components using the method according to the invention. This detection can be varied by making additional attachments of nucleic acids, for example to form triple DNA, or the additional incorporation of complexing molecules in double or triple helices accessible to measurement as binding events (claim 14). For this complexing or incorporation, use may advantageously also be made of metal complexes which bring about a particularly intensive electrical change in the field close to the electrodes.

The measurement principle, and the change in the electric field, make it possible in principle to distinguish the structure and nature of molecules by means of quantitative analysis of the impedance spectrum. Differentiation according to type and size of the molecules is possible through quantitative evaluation and, in particular, by calibration of the impedance spectra using known molecular species.

The invention will be explained below with reference to several figures and an **example**.

Figure 1 shows possible arrangements of the ultra-microelectrode arrays;

Figure 2 shows the adsorption of SH-biotin;

Figure 3 shows Nyquist plots of an electrode modified with SH-biotin and one additionally complexed with streptavidin;

Figure 4 shows the amperometric section of p-aminophenol.

Figure 1 shows various possible arrangements of ultra-microelectrode arrays. In this case

1a is a parallel arrangement in the form of strips;

1b is a parallel arrangement in the form of meanders;

1c is a finger-like interdigital arrangement;

1d is a circular parallel arrangement;

1e is a circularly stacked and mutually insulated arrangement;

Very much like the arrangement in Figure 1d is the arrangement of the electrodes as coils running parallel.

The mutually insulated ultra-microelectrodes 1 and 1', with their contacts to the electrical connection 2 and to the insulation layers (for example silicon nitride) 3 on the chip are arranged on a planar support (for example a silicon chip) 4. In the multilayer arrangement in Figure 1e, the electrode

plane 1 is insulated from the electrode plane 1' by intermediate insulation 5.

Illustrative embodiment

An interdigital gold electrode array, structured according to **Figure 1c**, has an electrode width of 1 µm and an electrode spacing of 0.7 µm. The electrodes are modified with a 1 ml, 10 mmol/l SH-biotin solution by means of self-assembling.

Figure 2 represents the adsorption of 10 mmol/l SH-biotin in a 0.1 mol/l sodium buffer solution as a capacitance/time plot for an applied potential of 50 mV and an additionally imposed amplitude of 10 mV at a pair of interdigital gold electrodes. The electrode capacitance decreases after the addition of SH-biotin to the solution. After about 2000 seconds, the surface of the gold is fully covered with -S-biotin. After 10 min of washing the electrode in 0.1 mol/l sodium buffer solution, the adsorbed monomolecular molecular layer is complexed in a subsequent step with streptavidin by dipping the modified electrode for 2 hours in a 50 U/ml solution. After the β-galactosidase-streptavidin modification, the electrode was rinsed for 10 min in 0.1 mol/l sodium buffer solution and subsequently secured in a measuring cell.

Figure 3 shows so-called Nyquist plots for a

potential of 50 mV, an amplitude of 10 mV and a frequency range of between $2x10^{-3}$ Hz and $1x10^{6}$ Hz, measured as two-pole impedance. Curve I represents the electrode modified with SH-biotin, and curve II the same electrode after additional complexing of the SH-biotin with β-galactosidase-streptavidin. The change in the impedance shows the disturbance of the dielectric between the electrodes by the complexed molecule, and further represents completed binding between the biotin and the streptavidin-enzyme complex.

The enzyme β-galactosidase on streptavidin is used independently as combined amperometric detection of the binding of the β-galactosidase-streptavidin to the SH-biotin. This detection is carried out with the function of the β-galactosidase, the enzymatic conversion of 5 mmol/l p-aminophenyl-β-D-galacto-pyranoside (p-APG) to p-aminophenol, by means of an amperometric oxidation-reduction of the p-aminophenol.

Figure 4 shows the amperometric detection of p-aminophenol on the same electrodes with an oxidation potential of 250 mV and a reduction potential of -50 mV relative to an Ag/AgCl reference electrode, after the addition of 5 mmol/l p-APG in 0.1 mol/l sodium buffer solution to the measuring cell. The continuous conversion of p-APG to p-aminophenol, which is represented by the linear rise in the current, indicates that the enzyme increases the p-aminophenol concentration in the measuring chamber.

Claims

1. Method for the detection of molecules or molecule complexes,

- a sample to be measured being brought into contact with an ultra-microelectrode arrangement which has at least two electrode structures that are arranged relative to one another such that the distances between the various structures lie in the ultra-micro range,
- an alternating electric field being produced by application of an electric potential, and
- the changes in current or potential, which are caused by species present or created in the sample to be measured, being measured.

2. Method according to claim 1, in which the field changes are measured using impedance spectroscopy.

3. Method according to claim 1 or 2, in which the detuning of the electric field, which is caused by species present or created in the sample to be measured, is measured independently of time or as a function of time by measuring the capacitive and/or resistive components and/or the phase angle.

4. Method according to one of claims 1 to 3, in which the molecules or molecule complexes are detected by virtue of their binding or attachment or diffusion.

5. Method according to one of claims 1 to 4, a plurality of electrode arrangements being stacked, and

the crossover points being insulated from one another by insulation layers, and the measurements being taken sequentially, in parallel or simultaneously.

6. Method according to one of claims 1 to 5, characterized in that the alternating electric field is superimposed or excited with a direct-current component.

7. Method according to claim 6, amperometric oxidations or reductions or redox recycling of molecules having electrically active groups or of redox mediators being measured in the sample to be measured.

8. Method according to one of claims 1 to 7, in which species to be measured self-assemble on the active electrode surfaces and are measured in the bound state.

9. Method according to one of claims 1 to 8, in which molecules are bound on the electrode surfaces by electropolymerization and are measured in the bound state.

10. Method according to one of claims 1 to 9, molecules being fixed in the gaps between electrodes and/or on the entire surface of the electrodes by physical or chemical binding, and being measured.

11. Method according to one of claims 8 to 10, a first fixed molecular layer containing a bonding group which, itself or through a difunctional reagent, binds a second molecular layer, which may in turn bind

others, and these events or their reverse being measured.

12. Method according to claim 11, in which the first molecular layer contains complexing groups which bind their complementary binding partner these events or their reverse being measured.

13. Method according to claim 11 or 12, in which the first molecular layer is a deoxiribonucleic-acid or ribonucleic-acid component which binds a complementary molecule strand by hybridization, this event or its reverse being measured.

14. Method according to claim 13, in which the molecular arrangement binds a further nucleic acid component or a complexing or intercalating molecule, and this event or its reverse is measured.

15. Method according to one of claims 1 to 14, in which the molecules or molecule complexes are detected in that they differ by size and/or type.

16. Method according to one of claims 1 to 15, in which the active electrode surfaces consist of gold, platinum, iridium or other noble metals, of carbon materials or of other conductive materials or of combinations thereof.

17. Method according to one of claims 1 to 16, in which the electrodes are applied to, or are incorporated in, silicon compounds, glass, ceramic, organic polymers or other insulating materials.

18. Method according to one of claims 1 to 17, in which, by coating on a substrate or embedding in such, the electrodes are arranged as bands or strips or circular structures or interdigital arrangements in the micrometer or submicrometer separation from one another.

19. Method according to one of claims 1 to 18, in which at least some of the electrodes are arranged as multilayer structures that are insulated from one another and, if appropriate, intersect.

20. Method according to one of claims 1 to 19, in which the active electrode surfaces can have direct and/or alternating current applied to them, individually or in groups, via insulated supply leads and/or electronic components.

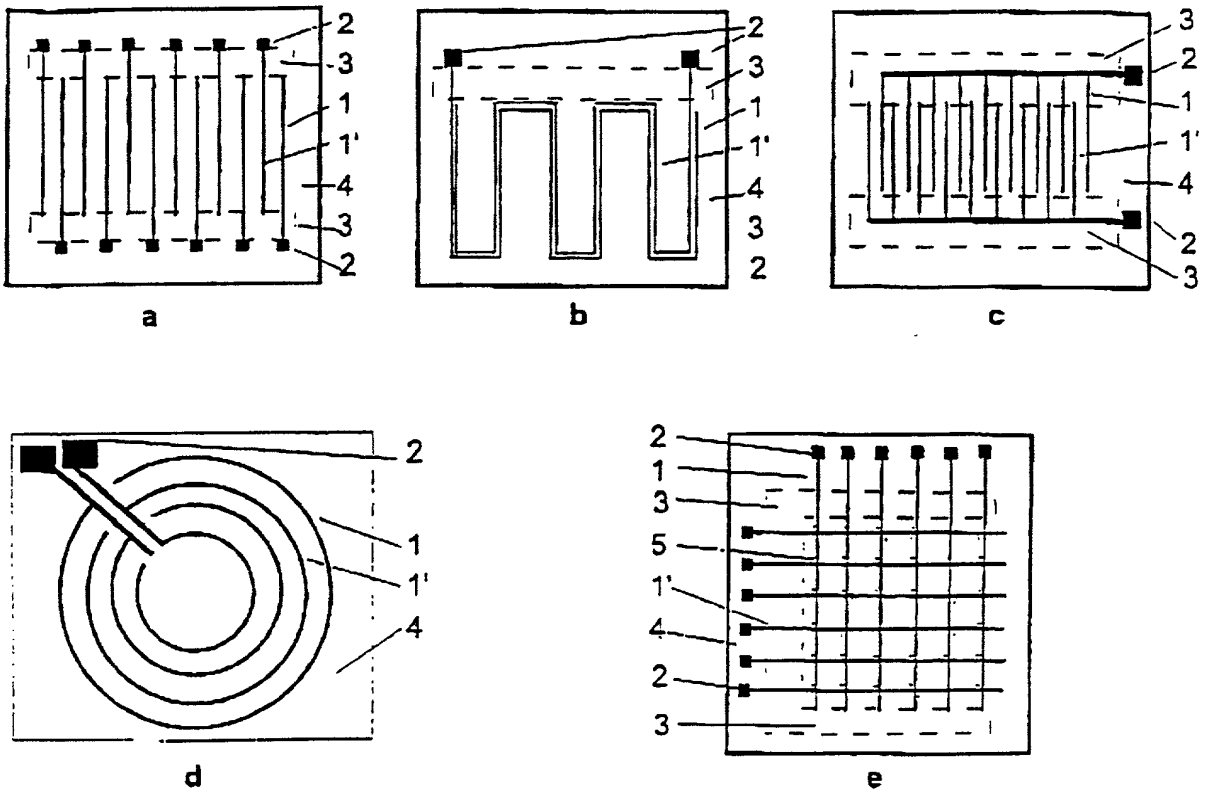
a
b
c
d
e

Fig. 1

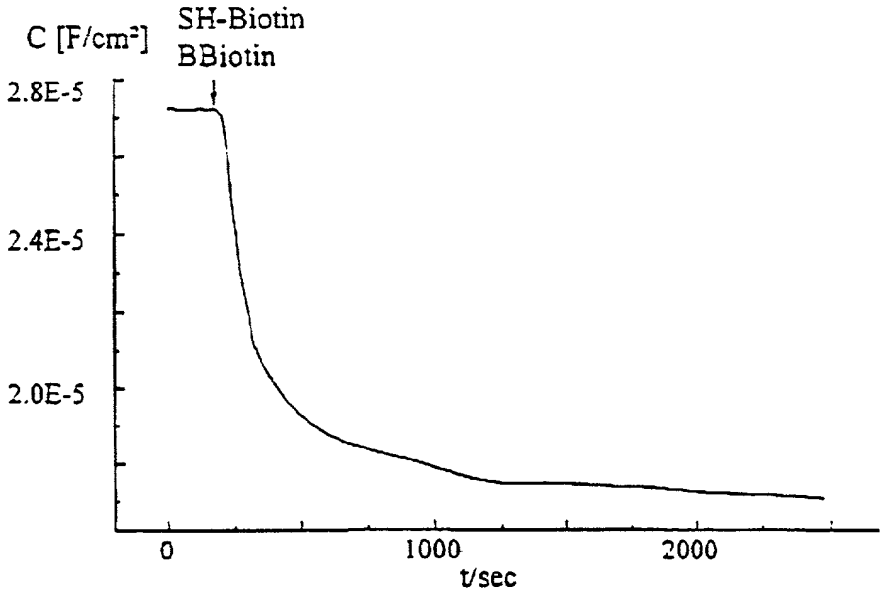
C [F/cm²]
SH-Biotin
BBiotin
2.8E-5
2.4E-5
2.0E-5
0
1000
2000
t/sec

Fig. 2

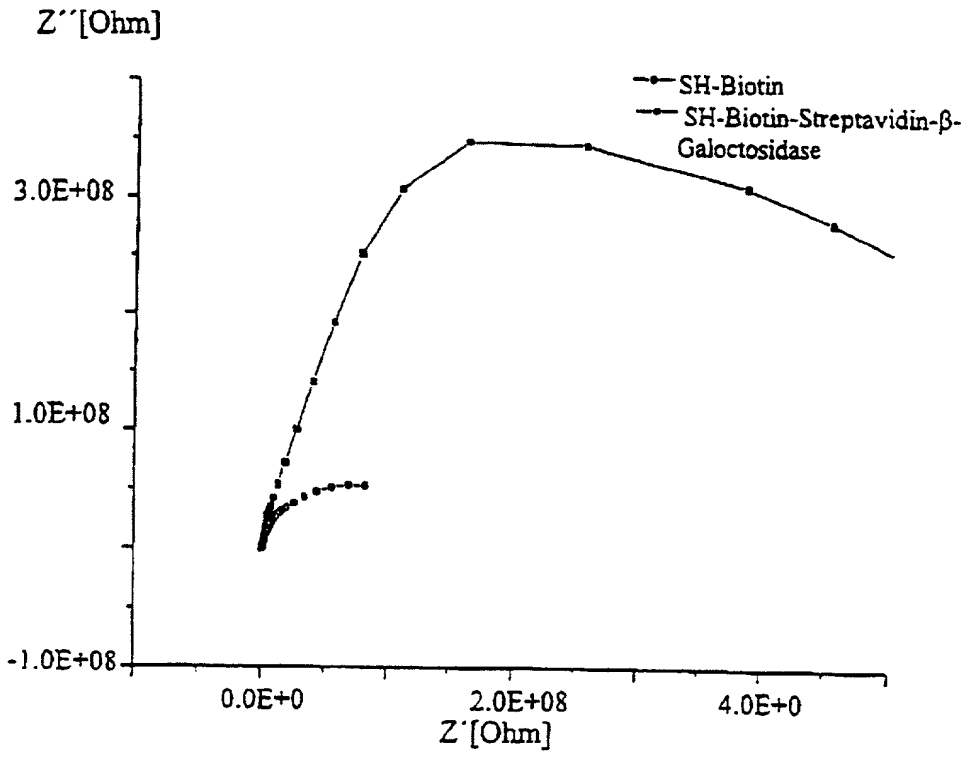
Z´´[Ohm]
SH-Biotin
SH-Biotin-Streptavidin-β-Galoctosidase
3.0E+08
1.0E+08
-1.0E+08
0.0E+0
2.0E+08
4.0E+0
Z´[Ohm]

Fig. 3

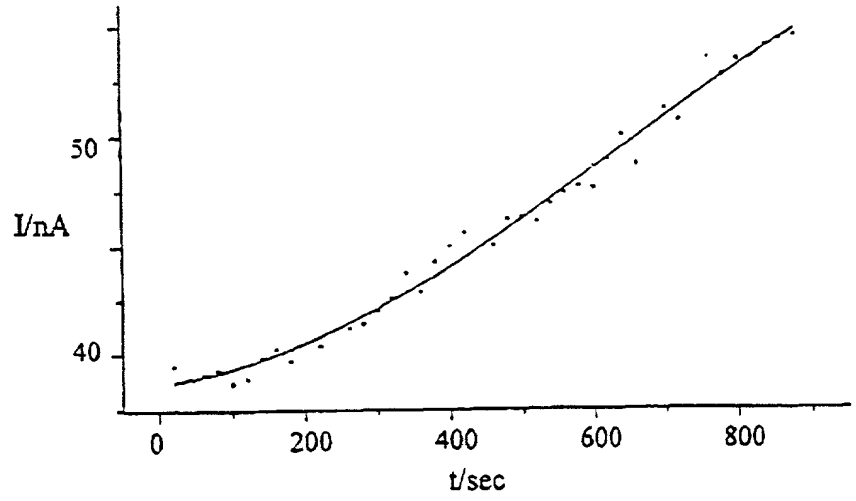
50
I/nA
40
0
200
400
600
800
t/sec

Fig. 4

Docket No. 60953/119

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

DETECTION OF MOLECULES AND MOLECULE COMPLEXES

the specification of which is attached hereto unless the following box is checked:

☒ was filed on ***March 12, 1997*** as United States Application Number or PCT International Application Number ***PCT/DE97/00494*** and was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
196 10 115.8	*Germany*	*14 March 1996*	*yes*

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

APPLICATION NO.	FILING DATE

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; William T. Ellis, Reg. No. 26,874; John J. Feldhaus, Reg. No. 28,822; Patricia D. Granados, Reg. No. 33,683; John P. Isacson, Reg. No. 33,715; Donald D. Jeffery, Reg. No. 19,980; Eugene M. Lee, Reg. No. 32,039; Richard Linn, Reg. No. 25,144; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Sybil Meloy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 28,665; Charles F. Schill, Reg. No. 27,590; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115; Harold C. Wegner, Reg. No. 25,258.

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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